

FABRICATION AND ANTIMICROBIAL ANALYSIS OF COMPOSITE
BIODEGRADABLE FILM FROM BREADFRUIT

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ABSTRACT

This study will be focusing on fabrication and antimicrobial analysis of composite biodegradable film from breadfruit starch-chitosan. The films were characterized in terms of physical, chemical and also the antimicrobial analysis. The antimicrobial effect of composite films was tested on *Bacillus subtilis* (*B.subtilis*) and *Escherichia coli* (*E.coli*) represent Gram-positive bacteria and Gram-negative bacteria respectively. The sample was prepared by cutting each sample into 2cm x 2cm and were placed into solid media that bacterial growth plate. Inhibition of bacterial growth was examined using zone inhibition method on solid media. The composite biodegradable film were characterized by fourier transform infrared spectroscopy (FTIR) analysis by preparing the sample with 1cm x 1 cm and revealed the spectrum of composite film containing chitosan and breadfruit starch at peak shifted from 3100 cm^{-1} to 3400 cm^{-1} due to hydroxyl stretching interactions. The amino acid group peak of chitosan at bands 1650.40 cm^{-1} . The composite biodegradable film also undergoes scanning electron microscopy (SEM) to characterize the morphology of the films with scan area of 0.3cm x 6cm. The cross section of the composite film shown distinctive film structure. Furthermore, water solubility also tested by cutting the each sample into 5cm x 5 cm and were dried at 70°C in a vacuum oven for 24 hours to constant weight. The sample films was immersed into 20ml of distilled water and placed into 50ml screw cap tubes and gently shaking for 24 hours . The result expose the decreasing percentage of water solubility by the addition of chitosan. Tensile strength (TS) and Elongation (*E*) break also tested and the result shows that TS inversely proportional to *E*. Degradation analysis also expose the film degrade under soil condition. Moreover, the characterization of breadfruit flour also characterized in terms of their structure.

ABSTRAK

Kajian ini berfokus pada fabrikasi dan analisis antimikrob terhadap filem komposit dari Sukun-kitosan pati. Filem komposit ini dikaji dari segi fizikal, kimia dan juga analisis antimikrob. Kesan antimikrob dari filem komposit diuji pada *Bacillus Substilis* (*B.substilis*) dan *Escherichia coli* (*E.coli*) yang masing-masing merupakan bakteria Gram-positif dan bakteria Gram-negatif. Sampel disediakan menjadi 2cm x 2cm dan ditempatkan ke dalam media pepejal yang mengalami pertumbuhan bakteria. Perencatan pertumbuhan bakteria telah diteliti dengan menggunakan kaedah zon perencatan pada filem komposit. Filem komposit juga diuji dengan spektroskopi inframerah (FTIR), sampel disediakan dengan 1cm x 1cm dan diuji untuk mengenalpasti kehadiran kumpulan berfungsi dalam filem komposit dan kumpulan hidroksil dikenalpasti pada puncak 3100 cm^{-1} hingga 3400 cm^{-1} . Kumpulan asid amino pada kitosan di puncak $1650,40\text{ cm}^{-1}$. Morfologi permukaan juga dikaji melalui mikroskopi pengimbas elektron (SEM) dan sampel filem disediakan dengan kawasan 0.3cm x 6cm. Bahagian melintang filem komposit menunjukkan struktur filem yang berbeza. Selanjutnya, kelarutan air juga diuji dengan memotong setiap sampel ke cm 5cm x 5 dikeringkan pada 70°C dalam oven selama 24 jam untuk berat malar. Kemudian, sampel direndam dalam 20ml air suling dan ditempatkan ke dalam balang 50ml dan digoncang secara konsisten selama 24 jam. Peratus kelarutan dan hasilnya menunjukkan peratusan penurunan kelarutan dalam air dengan penambahan kitosan. Kekuatan tarik (TS) dan Pemulihan (*E*) istirahat juga diukur dengan menggunakan alat uji mekanik universal dan hasilnya menunjukkan TS berkadar songsang dengan *E*. Degradasi analisis juga menunjukkan filem komposit mengalami penyusutan berat selepas beberapa hari di bawah tanah. Selain itu, ciri-ciri tepung sukun juga dikaji dalam struktur tepung.

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LIST OF ABBREVIATIONS

PEG	-	Polyethylene glycol
AM film	-	AntiMicrobial film
O ₂	-	Oxygen gas
HCl	-	Hydrochloric acid
NH ₂	-	Amide
NaOH	-	Sodium hydroxide
OH	-	Hydroxyl group
CHO	-	Aldehyde group
NH ₃ ⁺	-	Ammonium
C=O	-	Carbonyl group
TS	-	Tensile strength
<i>E</i>	-	Elongation at break
FTIR	-	Fourier Transform Infrared
SEM	-	Scanning Electron Microscope

LIST OF SYMBOLS

%	-	Percentage
g	-	gram
cm	-	centimetre

CHAPTER 1

INTRODUCTION

1.1 Background to the study

The breadfruit tree, *Artocarpus utilis* there is some confusion regarding the origin of the Breadfruit tree. In immature fruits fructose was the main sugar, but this was replaced by glucose and sucrose as maturation proceeded. Levels of Fe, Na, P, Ca and K were thought to have some nutritional importance due to its starchy content attempts have been made to utilize breadfruit flour as a source of dough for Western diets, in Nigeria, Olatunji and Akinrele (1978) examined the qualities of wheat flour that had been diluted with flours from certain tropical plants including breadfruit. They found that levels of non-wheat flour in excess of 10% of the total mix gave unsatisfactory results. Different results were obtained by Arceley and Graham (1984) who showed that bread, cakes and puddings containing high levels (up to 75%) of breadfruit flour were well accepted by members of the tasting panel in Puerto Rico, indicating the usefulness of breadfruit flour as a supplementary dietary product.

Polymers have been used in agriculture and horticulture since the middle of the last century. In the past, plasticulture (that is the use of plastics in agriculture) was introduced primarily in developed countries, but is recently spreading to developing

countries as well. Growth is particularly strong in areas with limited farmland such as in Europe, Japan and Korea (Briassoulis, 2007).

1.2 Identification of problem statement

A serious negative side effect associated with the steadily growing use of plastics in agriculture concerns the parallel growing disposal problem of thousands of tons of agricultural plastic wastes produced each year. Unfortunately, a large portion of these is left on the field or burnt uncontrollably by the farmers releasing harmful substances with the associated obvious negative consequences to the environment. Aesthetic pollution and landscape degradation of regions of natural beauty represent an additional negative environmental impact (Briassoulis 2007). The large production of non-biodegradable plastic-based waste has become a global problem in terms of both cost and ethics. Natural ingredients based on polysaccharides, proteins and lipids, extracted from plants, foods and animal tissues offer alternative packaging options that minimize environmental pollution at a relatively low cost. Applications of these ingredients in the food and pharmaceutical industries have been specified. Edible packaging could function as a barrier for water, oxygen and lipid transfer in food systems and therefore contribute to the extension in the shelf-life (Wang *et al.* 2009).

In the food packaging sector, starch-based material has received great attention owing to its biodegradability, edible, wide availability as agricultural surplus raw material, abundant, can be produced at low cost and at large scale, nonallergic, easy to use and thermoprocessable. Several studies are concentrated on the development of starch-based materials for the above-mentioned reasons. Starches are polymers that naturally occur in a variety of botanical sources such as wheat, corn, potatoes and tapioca. It is a renewable resource widely available and can be obtained from different left over of harvesting and raw material industrialization. Besides, the preponderance of amylose in starch gives rise to stronger films. However, biodegradable products based on starch, possess many disadvantages, mainly attributed to the water solubility, brittle nature of starch films and poor mechanical properties. In order to improve mechanical

properties and water resistance, starch can be modified by several methods such as blending with synthetic or natural polymers. One of the effective strategies to overcome the poor mechanical properties, while preserving the biodegradability of the materials, is to associate starch with chitosan (Salleh *et al.* 2007).

1.3 Research Objectives

In order to achieve the above objective, the following scopes have been identified:

- a. To fabricate composite biodegradable film from breadfruit starch-chitosan.
- b. To characterize composite biodegradable film in terms of morphology, physical and chemical properties.
- c. To analyze the antimicrobial activity of the fabricated biodegradable film.

Packaging is one of the most promising active packaging systems. Increase demand in food safety, quality, convenience and environmental concerns associated with the handling of plastic waste has emphasized the importance in developing biodegradable and edible films from natural polymers, such as starch. Starch-based film is considered an economical material for antimicrobial packaging. Chitosan has a widely been used in antimicrobial films, to provide edible protective coating, dipping and spraying for the food products due to its antimicrobial properties. chitosan act as antimicrobial agent into starch-based film enhance antimicrobial, physical and mechanical properties of starch-based film. The starch-based film also having antimicrobial properties that can extend shelf-life of the food packed. The antimicrobial effect of composite films was tested on *Bacillus subtilis* (*B.subtilis*) and *Escherichia coli* (*E.coli*) represent Gram-positive bacteria and Gram-negative bacteria respectively. Inhibition of bacterial growth might be able examined using on solid media and liquid culture test (optical density measurements). The control (pure wheat starch) and AM film (incorporated with chitosan and lactic acid) were produced by casting method (Salleh *et al.* 2007)

Films carrying food additives, such as antioxidants, antimicrobial agents, will be the developing tendency of functional food packaging in the future (Li *et al.*, 2006).

Antimicrobial films are allowed to contain higher concentrations of antimicrobial agents than that is permitted in food therefore, when food is packaged with antimicrobial film, the antimicrobial agents in the film are gradually released to the food surface and will remain there in a high concentration, which extends food shelf life and decreases the actual concentration of antimicrobial agents in the whole food (Shen *et al.* 2010).

1.4 Scope of study

The scope of study in this biodegradable films are:

- a. To study the fabrication of composite biodegradable film from breadfruit starch-chitosan.
- b. To study the characterization of composite biodegradable film using scanning electron microscopy (SEM), fourier transform infra-red (FTIR), water solubility of film, tensile strength (TS).
- c. To study effect of antimicrobial activity of composite biodegradable film by agar diffusion method (zone inhibition assay).
- d. To study the biodegradability of composite biodegradable film.
- e. To study the characterization of composite biodegradable film.

1.5 Significance and benefit of study

The significance and benefit of this study are:

- a. Facilitate to preserve food in food industries by its antimicrobial properties.
- b. Help to preserve the environment by producing competence biodegradable film.
- c. Help to store and preserve clinical equipment and maintain hygiene by its antimicrobial properties.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable film

Biodegradable is being able to be broken down by natural processes into more basic components and usually broken by bacteria, fungi, or other simple organism. It is also often associated with environmentally friendly product and it has been made from renewable and natural polymers such as starch (Bourtoom *et al.* 2008)

Biodegradable edible films and coatings based on protein resources physical properties and applications in food quality management (Arvanitoyannis, 2006).

Derived from natural sources, these biodegradable, biocompatible and non-toxic polymers meet the growing demands for environment-friendly products (Clarival and Halleux, 2005).

In this study, biodegradable films and composites for agricultural applications (greenhouse, walk-in tunnel, low tunnel covers, and mulching) are in the focus. Petroleum-based plastics often remain undegraded after discard and a time-consuming and uneconomical recycling is unavoidable. The adsoption of biopolymers avoids the removal of residual materials from the growing environment after functional compliance (Espí *et al.*, 2006 and Joo *et al.*, 2005).

The film forming and thermoplastic properties due to their abilities to form weak intermolecular interactions, i.e. hydrogen, electrostatic and hydrophobic bonds make, i.e. sodium caseinate a promising raw material for the production of biodegradable films and coatings (Audic and Chaufer, 2005).

An enzymatic catalyzed cross-linking of proteins associated with a controlled additive crystallization appears to be a promising way to improve physicochemical properties of biodegradable films and coatings. The application of a biodegradable film as barrier between fruits and vegetables and their surroundings is becoming an increasingly important venture because consumers demand of hygienic and sanitary products (Koide *et al.*, 2007).

Although biodegradable films are more expensive than the petrochemical materials, they will biodegrade into CO₂, water, and biomass under aerobic conditions, or methane and biomass under anaerobic conditions (Avella *et al.*, 2005).

The production of biodegradable and edible films from carbohydrates and proteins adds value to low-cost raw materials and can play an important role in food preservation (Ave'rous *et al.*, 2001, Krochta and Miller, 1997).

Several studies reported the use of starches from different sources to prepare films and coatings with different properties, and have indicated that these carbohydrates are promising materials in this regard (Ave'rous *et al.*, 2001, Larotonda *et al.*, 2005, Mali *et al.*, 2005).

2.2 Food Packaging

Food packaging is packaging for food to protect food from spoil. The materials most used for food packaging are the petrochemical-based polymers, due to their

availability in large quantities at low cost and favourable functionality characteristics, such as, good tensile and tear strength, good barrier properties to O₂ and heat sealability (Alves *et al.*, 2006) .

Usually it also contain of non-biodegradable which leading to serious ecological problem and cause of that the biopolymers commonly used to produce biodegradable films. Antimicrobial packaging is a form of active packaging. Active packaging interacts with the product or the headspace between the package and the food system, to obtain a desired outcome (Labuza, Rooney and Brody).

Antimicrobial food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself (Appendini, *et al.*, 2002).

The most successful commercial application of antimicrobial packaging has been sachets that are enclosed loose or attached to the interior of a package.

2.3 Composite

Composite made from two or more constituent materials with significantly different physical or chemical properties which remain separate and distinct on a macroscopic level within the finished structure. The surface morphology and conductivity of the PANI/SPSF composite film were characterized by environmental scanning electron microscopy and four-probe technique, respectively (Lu *et al.* 2008).

2.4 Chitosan

Chitosan is a biodegradable natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non-toxicity and mucoadhesion (Sinha *et al.* 2004).

A natural linear biopolyaminosaccharide is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose (Muzzarelli, 1977 and Roberts, 1992).

It also a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution ($\text{pH} < 6.5$), which can convert the glucosamine units into a soluble form R-NH_3^+ (Chandy and Sharma, 1990).

Chitosan is known to have good complexing ability; the $-\text{NH}_2$ groups on the chain are involved in specific interactions with metals. It has one primary amino and two free hydroxyl groups for each C_6 building unit. When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media (Rinaudo, 2006).

Chitosan is a cationic polysaccharide and a copolymer of β (1 \rightarrow 4) linked glucosamine and N-acetyl glucosamine. Since chitosan has reactive amino/hydroxyl groups, it can be subjected to chemical modification to obtain a derivative with the desired characteristics (Rekha *et al.*, 2009).

The biological properties of chitosan include biocompatibility, biodegradability, non-toxicity, hemostaticity, antitumoral and antiviral activity(Chandy *et al*, 1993).

Due to its biocompatibility, biodegradability and it sability to open intercellular tight junctions, chitosan may become a valuable excipient for oral drug delivery systems (Berradaa *et al.*, 2005).

Chitosan is a non-toxic, biocompatible, biodegradable, natural polysaccharide derived from the exoskeletons of crustaceans and insects. Chitosan's biodegradability, immunological activity and high viscosity make it an excellent candidate as a depot/adjuvant for parenteral vaccination (Zaharoff *et al.*, 2007).

2.4.1 Degree of acetylation of chitosan

The characterization of a chitosan sample requires the determination of its average. Various techniques, in addition to potentiometric titration (Rusu-Balaita *et al*, 2003).

It also have been proposed, such as IR, elemental analysis, an enzymatic reaction or UV. The fraction of $-\text{NH}_2$ in the polymer can be obtained by dissolution of neutral

chitosan in the presence of a small excess of HCl on the basis of stoichiometry followed by neutralization of the protonated $-\text{NH}_2$ groups by NaOH using pH or conductivity measurements (Rinaudo *et al.* 2006).

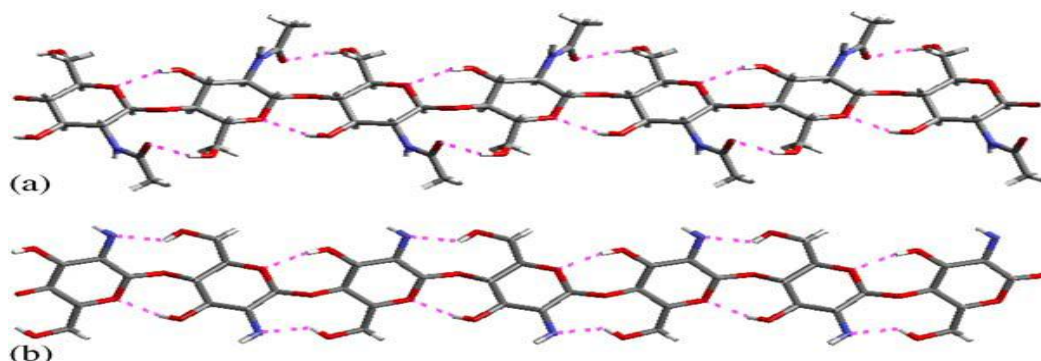


Figure 2.1 : Molecular modelling (a) of a chitin chain with two H—OH 3 and O 5, (2) between—OH 6 and O of CQO; and (b) of a chitosan chain with two H bonds (1) between—OH 3 and O 5, and (2) between—OH 6 and N.

2.5 Chitin

Chitin is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as aspergillus and mucor.. Chitin is a straight homopolymer composed of (1,4) linked N-acetyl-glucosamine units while chitosan comprises of copolymers of glucosamine and N-acetyl-glucosamine (Kas, 1997, Singla and Chawla, 2001, Kato *et al.*, 2003).

Chitin, a homopolymer comprising β -(1-4)-linked N-acetyl- glucosamine residues is one of the most abundant, easily obtained and renewable natural polymers, second only to cellulose. It is commonly found in the exoskeletons or cuticles of many invertebrates and in the cell walls of most fungi because of its high crystallinity, chitin is insoluble in aqueous solutions and organic solvents (Lason *et al.*, 2000).

2.6 Carbohydrate

Carbohydrate interactions play crucial roles in numerous biological processes (Grün *et al.*, 2006). It is a part of protein interactions are involved in a range of biological mechanisms, starting with fertilization and extending to pathologies. It also mediate diverse cellular activities, including cell recognition and growth control. Carbohydrate also contains of sugars and starches, which provide energy for humans and animals, and cellulose which make up many plant structures. The classifications of carbohydrate has three types which are monosaccharide, polysaccharide and oligosaccharide. The heavy B-chain possess the carbohydrate binding site (specific for galactose and N-acetylglactosamine) whereas the light A-chain is devoid of such sites. The B-chain can bind to cell surface glycolipids and glycoproteins having beta-1,4-linked galactose residues and facilitates the transport of the toxic protein (A-chain of ricin) into the cell (June *et al.* 2006)

2.6.1 Monosaccharide

Monosaccharides are a type of simple carbohydrate, or simple sugar. It is obviously differentiated from disaccharides and polysaccharides by the number of rings the chemical compound and it has a single ring carbohydrate and also classified as aldehyde or ketone depending on what type of carbonyl group is contained in the formula. An aldehyde has a carbon bonded between hydrogen and oxygen, while a ketone has a bond between carbon and oxygen. These are expressed in formulaic mode as aldehyde (-CHO) or ketone (-CO-). The interaction between saccharide (S) and electrolyte (E) in aqueous solution is of great importance in biochemistry and biophysics (Kelei, *et al.* 2000).

The density data for monosaccharide (d-xylose, d-arabinose, d-glucose and d-galactose) NaCl water systems, combined with data for the related binary systems, have been employed to calculate infinite dilution apparent molar volumes of the monosaccharides and NaCl and enables to obtain some information on the solvation of both electrolyte (ions) in aqueous monosaccharide and saccharide in aqueous NaCl solutions (Kelei, *et al.* 2000).

2.6.1 Polysaccharide

Polysaccharides are chains of sugar units that form in configurations from tens to thousands of units long. For example chitin is a polysaccharide that is a component of fungal cell walls, and forms the outer skeletons of insects and crustaceans. Regarding Pornsack., *et al.* (2006) statement two anionic polysaccharides, (sodium alginates and low methoxy pectins), that can react with divalent cations (e.g. calcium ions) to form cross-linked and water-insoluble gels.

2.6.2 Oligosaccharide

Oligosaccharides are a diverse and complex class of compounds that are broadly distributed in nature. Their biological significance ranges from defined roles as structural elements and energy sources, notably cellulose and starch, to less defined roles as constituents of glycoproteins and glycolipids on mammalian cell surfaces and microbial metabolites (Varki 1993). Vicinal glycosylation has been shown to markedly affect the conformational properties of a branched oligosaccharide compared with its parent disaccharides (Jesus *et al.*, 1999).

2.7 Starch

Starch is one of the most commonly used raw materials to prepare biodegradable film, because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Maizura *et al.*, 2007).

Starch biosynthesis is a complex process. Sucrose (derived from photosynthesis) is the starting point for alpha-glucan deposition. In the cell cytosol the sucrose is

converted to uridine diphosphate glucose (UDP-glucose) and fructose by sucrose synthase, the UDP-glucose being subsequently converted to glucose-1-phosphate (G-1-P) in the presence of pyrophosphate (PP_i) by UDP-glucose pyrophosphorylase (Tester *et al.*, 2004).

Starch has unique thermal properties and functionality that have permitted its wide use in food products and industrial applications (Wajira *et al.*, 2008).

Starch, as an abundant raw material with low cost, has been applied in the field of degradable plastics, and blend films containing starch are potential materials in the agriculture, medicine, and packaging industries (Xiong *et al.*, 2008).

Starch blended with synthetic polymer polyvinyl alcohol (PVA) has been studied as a potential biodegradable polymer (Follain *et al.*, 2005; Xiao and Yang, 2006, Zhai *et al.*, 2002).

Depending on the degree of biodegradability, it seems that PVA might provide a stable support medium for starch films (Jayasekara *et al.* 2004).

Starch molecules play an important role in the functional properties of the whole starch, therefore correlations established between the thermal and functional properties of starches aid in understanding the potential relationships between the structural features of the starch molecules and their effects on more than one kind of functional behavior (Amalia *et al.*, 2005).

2.7.1 Amylose and amylopectin fine structure

Amylose and amylopectin have different structures and properties which have been discussed and reviewed by many authors. Amylose is a relatively long, linear α -glucan containing around 99% (1 \rightarrow 4)- α - and (1 \rightarrow 6)- α - linkages and differs in size and structure depending on botanical origin and amylose has a molecular weight of approximately 1×10^5 – 1×10^6 (Tester *et al.* 2004).

Amylopectin is a much larger molecule than amylose with a molecular weight of 1×10^7 – 1×10^9 and a heavily branched structure built from about 95% (1 \rightarrow 4)- α - and 5% (1 \rightarrow 6)- α - linkages (Tester *et al.* 2004).

2.8 Antimicrobial

Antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects. Antimicrobial polymers can be used in several food related applications including packaging. One is to extend the shelf-life and promote safety by reducing the rate of growth of specific microorganisms by direct contact of the package with the surface of solid foods. Second, antimicrobial packaging materials could be self-sterilizing or sanitizing. Such antimicrobial packaging materials greatly reduce the potential for recontamination of processed products and simplify the treatment of materials in order to eliminate product contamination. Antimicrobial activity of silver is much higher than other metals, such as mercury, copper, lead, chromium and tin also the development of antibiotic resistant bacteria stains issue has resulted a new era for the revival of the long well-known antibacterial properties of silver and silver ions by the way the antibacterial mechanism of silver and its ions involves the interaction with the thiol groups of protein molecules present inside or outside the cell membrane and binds the bacterial cell membrane which inhibiting the replication capacity of DNA molecule, thereby affecting the cell viability (Vimala *et al.* 2010).

2.9 Polyethylene glycol (PEG)

Polyethylene glycol (PEG) is widely used as a covalent modifier of biological macromolecules and particulates as well as a carrier for low molecular weight (Samuel 1995). In addition Particular attention is paid to the comparative attributes of various reactive PEG derivatives, properties of the linkages formed, and possible side reactions (Samuel 1995).

Polyethylene glycols (PEGs) are high molecular weight polymers of ethylene oxide and also are designated by a number that roughly represents the average molecular weight of the polymers (Hermansky *et al.* 1995).

2.10 Acetic acid

Regarding Kyoko *et al.*, (2007) statement, acetic acid promoted water absorption of amylopectin in rice starch. As the concentration of acetic acid increased, the values of maximum, minimum, final viscosity, and consistency decreased, and that of breakdown increased (Kyoko *et al.*,. 2007).

CHAPTER 3

METHODOLOGY

3.1 Materials

Breadfruit was obtained from a local farmer (Bertam Malim Melaka, Malaysia). Acetic acid that used to dissolve chitosan was purchased from Fluka Chemical (Malaysia). Chitosan and Polyethylene glycol (PEG 400) was purchased from Sigma-Aldrich (Malaysia).

3.2 Flour Preparation

The breadfruit was cleaning and peeling. After that, it was ground into slurry with the addition of water. Then, the slurry was filtered through filter cloth and the filtrate was settled over night without shaking to recover the sediment as starch. The starch was dried at 40°C in oven around 4 hours. Finally, the breadfruit flour was produced.

3.3 Isolation of starch

To isolate the starch, 2g of breadfruit flour was taken and add with 2 ml of water. After that, the solution was heated and stirred by magnetic stirrer until the solution was gelatinized.

3.4 Chitosan solution preparation

The chitosan was weighed for 1g, 2g and 3g of three sample. 100 ml of 1% acetic acid was prepared and mix together with 1g of chitosan. Then, heat to 30°C and stirred around 300 rpm constantly by using stirrer until the solution seem like gel mixture.

3.5 Film preparation

The starch and chitosan solution was mixed together. The mixture was heated and still stirred with 300 rpm. To prepare the antimicrobial film, chitosan was added at different concentrations (1,2 and 3 g/100 g starch) in one time during the mixing period. Then, Polyethylene glycol (PEG 400) was added into the mixture and stirrer until the mixture miscible. Before antimicrobial agents were added into starch paste. When chitosan was incorporated into film, the pH of the gel-like mixture was 4.0 because of acetic acid used in preparing chitosan solution. The warm mixture was casted on framed glass plates, and then dried at 50 °C for 4 hours.